

AMENDMENT & RESPONSE UNDER 37 C.F.R. § 1.116 - EXPEDITED PROCEDURE

Serial Number: 09/820,198

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Dkt: 875.029US1

Title: METHODS TO PREPARE AND USE EPIDERMAL STEM CELLS

2. (Amended) A method to prepare isolated mammalian epidermal stem cells, comprising:
 - (a) providing [separating in] a sample comprising [a population of] mammalian epidermal stem cells, which sample is contacted with a dye with low or no cellular toxicity that binds DNA in viable cells and an agent that binds non-viable cells;
 - (b) separating the sample into a first population [in the sample which represents] of cells that are the smallest 30% of the cells in the sample and [which population comprises epidermal stem cells, from] a second population of cells that are larger than the cells in the [sample] first population, wherein the first population comprises a substantially pure population of epidermal stem cells; and
 - [(b)] (c) isolating a substantially pure population of epidermal stem cells from the smaller cells.
6. (Amended) The method of claim 1 or 2 wherein the [population of] sample comprises mammalian epidermal cells [is] which have been dissociated from non-epidermal cells [prior to separation].
7. (Amended) The method of claim 1 or 2 wherein the agent which binds DNA is a [population of mammalian epidermal cells is contacted with] Hoechst dye [prior to separation].
9. (Amended) The method of claim 1 or 2 wherein the agent that binds non-viable cells is [population of mammalian epidermal cells is contacted with] propidium iodide [prior to separation].
10. (Amended) The method of claim 7 wherein the agent that binds non-viable cells is [cells are further contacted with] propidium iodide [prior to separation].

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12. (Amended) The method of claim 1 or 2 wherein the [population of mammalian epidermal cells] sample is in a medium which lacks azide.
13. (Amended) The method of claim 1 or 2 wherein the sample [population of mammalian epidermal cells] is further contacted with a nuclear-retained label prior to separation.
14. (Amended) The method of claim 1 or 2 wherein the cells in the second population [that are not epidermal stem cells] have proliferative capacity.
16. (Amended) A method to prepare [isolated] mammalian epidermal stem cells, comprising:
- (a) contacting a population of mammalian epidermal cells comprising epidermal stem cells with an amount of a first agent under conditions effective for viable cells to retain the first agent;
 - (b) contacting the population of (a) with an amount of a second agent under conditions effective for non-viable cells to retain the second agent; and
 - (c) separating the population of (b) into a first population of [which] cells which first population represents the smallest 30% of the cells which comprise viable epidermal stem cells and [at least one] a second population of cells which represents larger cells, which second population [that] does not comprise a substantial portion of epidermal stem cells.
21. (Amended) The method of claim 16 wherein the sample comprises [population of] mammalian epidermal cells which have been [is] dissociated from non-epidermal cells [prior to separation].
22. (Amended) The method of claim 16 wherein the [population of mammalian epidermal cells of step (a) is contacted with] first agent is a Hoechst dye.

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25. (Amended) The method of claim 16 wherein the sample [population of mammalian epidermal cells] is further contacted with a nuclear-retained label prior to separation.
26. (Amended) The method of claim 16 wherein the cells [that are not epidermal cells] in the second population have proliferative capacity.
39. (Amended) A method to prepare a substantially pure population of mammalian epidermal stem cells, comprising: separating a sample comprising [a population of] mammalian epidermal stem cells and epidermal cells which are not epidermal stem cells, which sample is contacted with a Hoechst dye and propidium iodine or pyronidine iodine, [which population comprises epidermal stem cells,] into a substantially pure population of epidermal stem cells and into at least one population of cells that does not comprise a substantial portion of epidermal stem cells.
40. (Amended) A method to prepare mammalian epidermal stem cells, comprising: separating a sample comprising [a population of] mammalian epidermal stem cells and epidermal cells which are not epidermal stem cells, which sample is contacted with a Hoechst dye and propidium iodine or pyronidine iodine, [which population comprises epidermal stem cells,] into a population which represents the smallest 30% of the cells in the sample and into a population which comprises larger cells, wherein the population with the smallest 30% of the cells comprises a substantially pure population of epidermal stem cells.

Remarks

Reconsideration and withdrawal of the rejection of the claims, in view of the remarks and amendments herein, is respectfully requested. Claims 1-2, 6-7, 9-10, 12-14, 16, 21-22, 25-26, and 39-40 are amended. Claims 1-40 are pending. The amendments to the claims are intended to further prosecution and are not intended to concede to the correctness of the Examiner's